therapeutics and serves as the basis of many diagnostic techniques. Force-based ligand detection offers many advantages over conventional approaches based on the thermodynamics of the interaction. It is label-free and permits crowded ambients. However, it requires very expensive equipment as well as labor intensive and time-consuming protocols. More severely, since force spectroscopy is inherently limited by thermal fluctuations, a molecular complex under investigation has to be probed thousands of times in order to achieve sufficient force resolution. This confines its application to low throughput formats. Here we present a high-throughput force spectroscopy approach in a parallel format, which in addition allows the detection of subtle changes in mechanical stability below the ones e.g. caused by a single base-pair mismatch in dsDNA. A very low affinity ATP selective DNA aptamer was implemented into a microarray compatible differential force detector design, wherein the relative stability of an aptamer·ligand complex is probed against a constant dsDNA reference complex. We found that the label-free assay selectively quantifies the concentration of ATP and that it reliably operates in a challenging, molecular crowded environment. The simplicity of the assay qualifies it as a tool that can be used in any laboratory equipped with basic fluorescence microscopy.

2152-Pos Board B122

Visualization of Force-Mediated Looping Dynamics of a Single DNA Molecule by the E. coli protein FIS

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Various experiments have suggested that DNA is ''looped'' by proteins, such as the E coli nucleoid associated protein FIS, in a concentration- and force-dependent manner. However, there has been no direct evidence that discrete DNA condensation domains are formed by FIS, until now. Using a combined magnetic tweezers/fluorescence microscopy apparatus, we have measured the dynamics and visualized the formation of discrete condensation domains in a single DNA molecule by FIS. Visualization was achieved by binding a gfp-FIS conjugate to lambda-DNA at concentrations higher than 1uM. The force dependence and rate of condensation are demonstrated.

2153-Pos Board B123

Distinguishing Dual DNA Binding Modes of Actinomycin D using Optical Tweezers

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Actinomycin D (ActD), the first antibiotic which exhibited anti-tumor activity, was initially believed to bind double stranded DNA (dsDNA) through intercalation. Later it was shown to bind single stranded DNA (ssDNA) with an order of magnitude higher affinity. ssDNA binding can be extremely important in inhibiting replication of viruses that replicate through ssDNA templates such as HIV. While these two binding modes can be separately quantified by studying binding to specific substrates, it is very difficult to determine the mode of binding to polymeric DNA. DNA stretching studies can precisely quantify intercalation by measuring the increase in DNA length upon intercalation. However, ssDNA binding also increases DNA length. Therefore, we have developed a method that combines the measured increase in DNA length with the overall DNA melting free energy change, allowing us to simultaneously determine ssDNA binding and intercalation as DNA is stretched. Using this method, we were able to distinguish between dual binding modes of ActD. We determined that the ssDNA binding of ActD ($K_{ss} \sim 10^8\ M^{-1}$) is much higher than its binding to dsDNA ($K_{ds} \sim 10^6\ M^{-1}$) for long polymeric DNA. We also determined the ssDNA and dsDNA binding site size, which are 3 bases and 6 base pairs, respectively.

2154-Pos Board B124

Inducer Effects on Lac Repressor-Mediated DNA Loops: Single-Molecule FRET Studies

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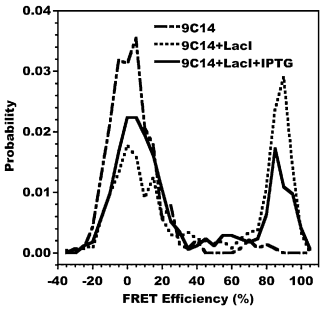
The *Escherichia coli* LacI protein represses the *lac* operon by blocking transcription. Tetrameric LacI binds simultaneously to a promoter-proximal DNA operator and an auxiliary operator, and the resulting DNA loop increases the efficiency of repression. A hyperstable closed-form LacI-DNA loop was previously shown to be formed on a DNA construct (9C14) that includes a sequence-directed bend flanked by operators. Previous bulk and single molecule fluorescence resonance energy transfer (SM-FRET) experiments on dual fluorophore-labeled 9C14-LacI loops demonstrate that LacI-9C14 adopts a single, stable, rigid DNA loop conformation, despite the presence of flexible linkers in LacI. Here, we characterize the LacI-9C14 loop by SM-FRET as a function of inducer isopropyl-β,D-thiogalactoside (IPTG) concentration. Energy transfer measurements reveal partial but incomplete destabilization of loop formation by IPTG, with no change in the energy transfer efficiency of the remaining looped population.



Models for the regulation of the *lac* operon often assume complete disruption of LacI-operator complexes upon inducer binding to LacI. Our work shows that even at saturating IPTG there is still a significant population of LacI-DNA complexes in a looped state, in accord with previous in vivo experiments that show incomplete induction.

2155-Pos Board B125

DNA Structure Selectivity of *Escherichia coli* versus *Thermus aquaticus* DNA Polymerase I

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Understanding substrate selection by DNA Polymerase I is important for characterizing the balance between DNA replication and repair for this enzyme *in vivo*. Due to their sequence and structural similarities, Klenow and Klentaq, the ''large fragments'' of the Pol I DNA polymerases from *Escherichia coli* and *Thermus aquaticus*, are considered functional homologues. We have examined the DNA binding thermodynamics of Klenow and Klentaq to different DNA structures: single-stranded DNA (ss-DNA), primer-template DNA (pt-DNA), and double-stranded DNA (ds-DNA). The DNA binding affinity trend for Klenow from weakest to tightest binding is ds-DNA < pt-DNA < ss-DNA. This is in contrast to Klentaq's DNA binding trend: ss-DNA < pt-DNA ≈ ds-DNA. Both Klenow and Klentaq released more ions when binding to pt-DNA and ds-DNA than when binding to ss-DNA in KCl buffer. ΔCp is the temperature dependence of the enthalpy of a reaction. Both of these non-sequence specific binding proteins exhibit relatively large heat capacity changes (ΔCp) upon DNA binding. ΔCp values for binding of Klenow and Klentaq to the different DNA structures do not follow the same patterns as the ΔG values for binding, suggesting the balance of electrostatic versus hydrophobic interactions in the binding interfaces also differ between the two species of polymerase. It is also found that Mg^{2+} significantly shifts the ds-DNA binding affinity of Klenow, but not Klentaq. Mg^{2+} may be shifting the partitioning between the polymerization and editing sites on Klenow. The differences in DNA structural selectivity of the two polymerases suggest that the *in vivo* functions of these two supposedly homologous polymerases are different, and that Taq polymerase is more likely to be involved in ds-break repair and end-preservation *in vivo*. Funded by the NSF and the Louisiana Biomedical Research Network.

2156-Pos Board B126

Modeling The Behavior Of DNA-Loop-Extruding Enzymes

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Condensin proteins are large complexes belonging to a family of ATP hydrolyzing proteins known as SMC (Structural Maintenance of Chromosomes). Condensins are believed to play a vital role in chromosomal assembly and segregation in eukaryotic cells but the details of their function along chromatin are poorly understood. Here, we propose a model to describe the behavior of DNA-loop-inducing proteins, such as type I restriction enzymes, which we believe can be used to understand condensin's function. We assume an effective motor behavior for these enzymes in which the bias of the two dimer heads is to travel away from each other, which results in loop formation along the DNA lattice. Processivity causes the enzymes to stack on top of each other. We further discuss the results of theory and computer simulations for different values of motor bias and processivity.

2157-Pos Board B127

Klenow and Klentaq-DNA Binding: the 'Glutamate Effect' is Primarily an Osmotic Effect

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DNA binding by Klenow (*E.coli*) and Klentaq (*T.aquaticus*) DNA polymerases has been studied as a function of monovalent salt concentration, pH and osmotic stress. We previously showed that DNA binding resulted in the net release of 4.5~5 ions from Klenow and 3~3.5 ions from Klentaq. Here, we report

that Klenow and Klentaq have minimal sensitivity to pH changes, with proton linkages of ~0.06 and ~0.3 respectively in KCl. Furthermore, osmotic stress data in KCl indicates 500~600 waters are released upon binding by both polymerases.
Glutamate is the major intracellular anion accumulated in *E.coli* in the presence of KCl in the external environment. The 'glutamate effect' is primarily characterized by an increase in DNA binding affinity when chloride is replaced by glutamate. Some proteins also exhibit decreased ionic linkage in glutamate. Klenow exhibits both aspects of the 'glutamate effect'. Substituting glutamate for chloride reduces the ionic linkage for Klenow by >50%. The presence of glutamate also increases the proton linkage of Klenow five fold and decreases water release by ~70% to approximately 150 waters. The dramatic decrease in water release highlights the osmotic nature of the glutamate effect. Glutamate and chloride salts behave as ionic inhibitors of DNA binding but glutamate salts also exhibit an osmotic enhancement effect.
While Klentaq's DNA binding affinity is also enhanced by glutamate, its ionic and proton linkages are not altered. The osmotic enhancement is present for Klentaq but it is not as significant in the salt concentration range at which nanomolar Klentaq-DNA binding occurs. *E.coli* DNA-binding proteins might have evolved to bind tightly at higher salt concentration to utilize the glutamate effect while accumulating intracellular glutamate.

2158-Pos Board B128
Atomic Force Microscopy In Solution Shows Nucleosome Positioning By Excluding Genomic Energy Barriers
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Because of the importance of the nucleosomal organization in processes such as replication, transcription, DNA repair and recombination, understanding the role of DNA sequence on the chromatin organization is one of the main challenges in functional genomics. In this context, we have studied the positioning of reconstituted nucleosomes on genomic 400-900bp Saccharomyces cerevisiae and human DNA fragments, including two promoter regions, by coupling AFM imaging in liquid with a physical modeling of nucleosome formation energy based on sequence-dependent DNA bending properties. An important result coming out from these studies is the excluding role of high energy barriers that prevent nucleosome formation (nucleosome free regions) that contributes to the global nucleosome organization of the chromatin fiber by some ''parking phenomenon''. The investigation of a yeast and a human gene promoter regions, that are respectively positively and negatively regulated by a chromatin organization, confirms the existence of energy barriers as well as their major impact on the positioning of these nucleosomes.
Altogether, these results show that nucleosome positioning by genomic energy barriers has a main role in the nucleosomal array assembly and is likely to be a key to the understanding of chromatin mediated regulation processes.

2159-Pos Board B129
Rapid Formation And Breakdown Of Protein-mediated DNA Loops
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The Lactose Repressor protein (LacI) is a paradigm for the study of transcriptional regulation and protein-DNA interaction. LacI represses transcription of the Lac operon in E. Coli by binding to two distant operator sites and bending the intervening DNA into a DNA loop. Despite a wealth of knowledge on the biochemistry of this process, the details of the binding dynamics are still unresolved, and are the subjects of several lines of current investigations. We present Tethered Particle Microscopy (TPM) data on designed hyperstable loop-forming DNA constructs and find that LacI-mediated DNA loops form and break down on time scales of the order of minutes. This is in stark constrast to measurements in competition assays by Mehta et al[1], who report loop lifetimes of days for these constructs. We propose two possible explanations for the LacI-loop formation process that hamronizes these seemingly contradictory observations. Specifically, we propose that the loop-forming LacI tetramer is destabilized by binding to the DNA, and that therefore the primary loop breakdown process is a dissociation of the tetramer into two DNA-bound dimers, which is in contradiction to the prevailing model for this process. Alternatively, we discuss what assumptions have to be made to explain these experimental results purely in terms of dissociation of the tetramer from DNA. Namely, we need to assume an outsized effect of spatial operator orientation on loop formation rates and postulate that the protein is extremely inflexible.

1. Mehta, R.A. and Kahn, J.D. ''Designed hyperstable lac-Repressor-DNA loop topologies suggest alternative looop geometries.'' Journal of Molecular Biology 294 (1999), 67-77.

Virus Structure & Assembly

2160-Pos Board B130
Retrovirus and the Cytoskeleton: Insights Into the Mechanism for Viral Assembly
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The assembly and budding of a new virus is a fundamental step in retroviral replication. Yet, despite substantial progress in the structural and biochemical characterization of retroviral budding, the underlying physical mechanism remains poorly understood. In particular, the mechanism by which the virus overcomes the energy barrier associated with the formation of high membrane curvature during viral budding is unknown. Using atomic force- and transmission electron microscopy we find that both human immunodeficiency virus and Moloney murine leukemia virus remodel the actin cytoskeleton of their host cells and utilize the forces it generates to drive their assembly and budding. Highly dynamic actin-filamentous structures which varied in size over the duration of budding appeared to emanate from the assembled virion. These actin structures assemble simultaneously or immediately after the beginning of budding, and disappear as soon as the nascent virus is released from the cell membrane. Analysis of sections of cryo-preserved virus infected cells by transmission electron microscopy reveals similar actin filaments structures emerging from every nascent virus. Substitution of the nucleocapsid domain implicated in actin binding by a leucine-zipper domain resulted in budding of virus-like particles that was not accompanied by remodeling of the cell's cytoskeleton. Notably, budding of viruses carrying the modified nucleocapsid domains was an order of magnitude slower than that of the wild type. The results of this study show that retroviruses utilize the cell cytoskeleton to expedite their assembly and budding.

2161-Pos Board B131
Capsid Assembly in Small, Unenveloped Icosahedral DNA and RNA Viruses
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There are two distinct mechanisms for the assembly of viral capsids. (1). In some cases, such as DNA bacteriophage, protein-protein interactions are strong, and protein-nucleic acid interactions are weak. Under suitable conditions, the proteins assemble into a capsid spontaneously, and the nucleic acid is then driven into the capsid by an ATP-driven motor. (2) In other cases, such as some single-stranded RNA viruses, protein-protein interactions are weak, while protein-nucleic acid interactions are strong. The capsids of these viruses do not assemble in the absence of the genome, but viral assembly occurs spontaneously if both the nucleic acid and the protein are present; no energy source is required. We are investigating both of these processes, using coarse-grained molecular mechanics models. The simplest model treats the assembly of bacteriophage capsids using a truncated triangular pyramid to represent the asymmetric unit, with van der Waals forces to promote association. For this model, the size and structure of the aggregates is sensitive to the dihedral angle between subunits (i.e., to the angle at the peak of the pyramid). Over a small range of angles, a solution containing isolated subunits at submicromolar concentrations will quickly assemble into spherical aggregates with twenty subunits each, and these become icosahedral when the temperature is lowered; this corresponds to the formation of T=1 viral particles. We are exploring a number of issues for these model bacteriophage, including: conditions yielding T=3 and higher structures; assembly of asymmetric units and mature viruses from quasi-equivalent monomers; and the effects of model cations.

2162-Pos Board B132
Assembly of Viruses and the Pseudo Law of Mass Action
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The self-assembly of viral capsids is believed to obey the Law of Mass Action (LMA), this despite the fact that viral assembly is not a reversible process. We present a soluble model for irreversible capsid assembly, the ''Assembly-Line Model'' (ALM) and show that, in this model, viral assembly from a supersaturated solution of capsid proteins is characterized by a shock front that propagates in the assembly configuration space from small to large aggregate sizes. If this shock front is able to reach the size of an assembled capsid, then a transient state develops characterized by a ''pseudo'' LMA that would be difficult